Exploring the impact of gene mutation on the progression of multistage colorectal cancer

Wentao Wang

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Haiquan Qin

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Zigao Huang

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Jiankun Liao

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Yongjie Qin

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Baojia Li

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Hao Lai

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

Xianwei Mo (✉ moxianwei@gxmu.edu.cn)

Guangxi Cancer Hospital and Guangxi Medical University Affiliated Cancer Hospital

<https://orcid.org/0000-0002-7910-9556>

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Abstract

OBJECTIVE: This article focused on the key role that the cumulative mutations of somatic genes play during the progression of colorectal cancer from benign adenoma to highly malignant mucinous adenocarcinoma. Moreover, the article explains the reasons for the differences in the prognosis of the left-sided and right-sided colon cancer by comparing the number of mutations in the cases.

METHODS: In this study, tumor specimens from patients with different stages and different states of co-occurring colonic adenoma, colonic adenocarcinoma, and colonic mucinous adenocarcinoma were extracted and analyzed for gene mutations in different tumor development stages during the progressive evolution from normal tissue to mucinous adenocarcinoma by second-generation sequencing gene detection.

Conclusion: The number of somatic gene mutations showed an increasing trend during the progression from normal tissue to the mucinous adenocarcinoma with the highest malignancy. No difference in the underlying genetic level between the right-sided and left-sided colon cancer was observed. However, the prognosis of the right-sided colon cancer patients was worse than that of the left-sided colon cancer patients, suggesting that the longer growth cycle of the right-sided colon cancer may be the main reason for the difference of prognosis.

Introduction

According to the 2018 International Agency for Research on Cancer (IARC) statistics on global cancer incidence and mortality, colorectal cancer is currently the third most prevalent and the second most deadly malignancy in the world with an estimated 1.8 million new cases and 881,000 deaths per year (1). Current colorectal cancer treatments mainly include laparoscopic surgery, resection of metastatic disease (e.g. liver or lung metastases), radiotherapy, and neoadjuvant and palliative chemotherapy (2). Research on the molecular mechanisms of colorectal cancer in recent years have made rapid progress in molecularly targeted drugs as well as immunotherapy treatments, which provide more precise and effective treatments for colorectal cancer with rare mutations, i.e., treatment plan.

Carcinogenesis is a complex, multistep process that can be caused by mutant combinations of oncogenes or cancer suppressor genes, or the epigenetic changes of DNA (e.g., methylation). Previous models have described the process through which colonic adenomas becomes cancerous through the activation, loss of function, or mutation of key oncogenes and tumor suppressor genes (3; 4). Briefly speaking, tumorigenesis begins with normal epithelium, which transforms to adenoma, then progresses to carcinoma in situ, and eventually becomes a more aggressive and metastatic malignancy. Some researchers proposed that the key to the progression of adenoma from adenocarcinoma is the result of cumulative mutations in genes rather than the specific nature of the mutations or the chronological order of the mutations (5). Among the genes that have been studied to date, adenomatous polyposis coli

(APC), Kirsten-ras (K-ras), and (tumor protein 53)TP53 are considered to be particularly important in tumorigenesis and progression (3).

Previous studies of colon cancer have all compared the OS, PFS, DFS, etc. of the samples by cohort studies with multiple samples. In this study, a single patient with simultaneous malignancies in different bowel segments was selected and subjected to gene sequencing to investigate the effect of mutations on the tumor. This eliminates individual differences and factors that may bias the results, and allows for a more accurate description of the mutational changes in the tumor at different development stages.

Multiple primary malignancies (MPMs) are defined as the presence of two or more primary malignancies in the same organism. The classical diagnostic criteria proposed by Warren and Gates in 1932 are as follows: (i) histologic confirmation that both the primary and secondary tumors are malignant; (ii) both malignancies must be separated by normal mucosa; and (iii) the probability that the second primary malignant tumor being the metastasis from the first must be excluded. However, two special conditions should be specifically emphasized: (i) malignant tumors invading multiple organs are considered as single tumors; (ii) malignant tumors with different histology are considered as multiple carcinomas, regardless of the location of origin and whether the multiple loci are diagnosed simultaneously (6; 7). The prevalence of MPMs varies from 11.7–0.734% due to geographical differences and different diagnostic methods. Epidemiological studies have shown that MPMs are common in colorectal cancer (20.4%) (8).

Method

Two cases of multiple primary colorectal cancer were selected for this study. Case 1 had sigmoid adenoma, adenocarcinoma of the ascending colon, adenocarcinoma of the rectum, and mucinous adenocarcinoma of the transverse colon. Case 2 was diagnosed with the Lynch syndrome and had adenoma of the ascending colon, adenocarcinoma of the ileocecal region, and mucinous adenocarcinoma of the sigmoid colon. We extracted tissues from different intestinal segments separately and analyzed using the second-generation gene sequencing.

In this study, the high-throughput gene sequencing platform was used to perform deep sequencing on the exons, introns, promoters, or fusion breakpoints, and the coding regions of the 70-gene signature (where plasma free DNA detection combined the ER-Seq assay developed by the GenePlus Medical Laboratory) as well as to detect the four types of mutations of tumor genes (including point mutations, small indels, copy number variations, and fusion genes currently known). The results have been evaluated by the Ministry of Health Clinical Testing Center with a full score, providing the most comprehensive and reliable test results with both accuracy and comprehensiveness.

Result

Frequency of genetic mutations

By analyzing the data from the results of genetic testing in patient 1 (Fig 1), we concluded that tissues at all states had a large number of somatic mutation genes, and the number of somatic mutations showed an increasing trend from normal tissues to mucinous adenocarcinoma tissues. From all mutated genes, it was found that the same somatic mutations were present across sample types, and that the overlap of mutations in each sample showed an increasing trend (11.76% for normal tissue vs. adenoma tissue, 25% for adenoma tissue vs. adenocarcinoma tissue, and 27.06% for adenocarcinoma tissue vs. mucinous adenocarcinoma tissue). In case 2 (Fig 2), the normal tissue, ascending colon adenoma tissue, ascending colon mucinous adenocarcinoma tissue, and sigmoid mucinous adenocarcinoma tissue also showed a trend of increasing number of gene mutations with increasing tissue malignancy. However, we found no significant overlap of somatic gene mutations for each type of samples.

Key gene mutations

To determine the significant mutations in colonic malignancies, we compared tissue samples of different nature. It was found that the different tissues had common key gene mutations. APC gene mutations occurred in normal tissues as well as in all three lesion tissues, but only KRAS and TP53 gene mutations were observed in mucinous adenocarcinoma tissues (Fig 3).

Comparison of genes mutations in the left-sided and right-sided colon cancer

The number of somatic mutations in the ascending colonic mucinous adenocarcinoma tissue and the sigmoid mucinous adenocarcinoma tissue of case 2 were compared (Table 2). The number of somatic mutations was 120 in the ascending colonic mucinous adenocarcinoma tissue and 93 in the sigmoid mucinous adenocarcinoma tissue. Moreover, in the somatic gene mutations of the two malignancies, the highest mutation frequency was higher in the ascending colonic mucinous adenocarcinoma tissue than in the sigmoid mucinous adenocarcinoma tissue. Only one KRAS exon and two APC exons were mutated in the sigmoid mucus adenocarcinoma tissue, while three KRAS exons and three APC exons were mutated in the ascending colon mucus adenocarcinoma tissue. In case 1, the number of somatic mutations in the right-side colonic adenocarcinoma was less than the left-side colonic mucinous adenocarcinoma (Table 1).

We also performed second-generation gene sequencing analysis of two sets of normal tissues adjacent to the malignant tumor in case 2, and it was found that the number of genetic mutations and the type of mutated genes were identical in the normal tissues at both sites (Table 3).

Discussion

Research into the genetic origins of colorectal cancer has been in full swing. However, the ultimate mechanisms are not yet fully understood. Moreover, according to the latest version of the NCCN (National Comprehensive Cancer Network) guidelines, the use of targeted drugs has significantly improved the prognosis of patients. Therefore, studying the mechanism of the gene mutations of colorectal cancer has

a very positive effect not only on clarifying the pattern of disease onset, but also on the evaluation of treatment effects.

We compared the genetic characteristics of four different tissues (normal tissue, adenoma tissue, adenocarcinoma tissue, and mucinous adenocarcinoma tissue) in two cases, and the number of somatic gene mutations showed an increasing trend during tumor progression. Moreover, in case 1, we found that the overlap rate of gene mutations in each type of sample tissue also showed an increasing trend. Mutations in proto-oncogenes and tumor suppressor genes have a tremendous impact on tumor development, and further accumulation of gene mutations leads to cancer progression(9). Case 2 differed from case 1 in its microsatellite instability. Although there was also an increasing pattern in the number of somatic mutations, no trend of accumulation of mutations was observed. We can assume that the instability of microsatellite may have allowed the colon cancer to undergo multi-directional and multi-channel oncogenic mutations during the progression, thus showing non-cumulative mutations that do not overlap with each other.

Interestingly, we found that the APC gene mutations occurred in all four tissues, and KRAS and TP53 gene mutations occurred simultaneously in the more aggressive mucinous adenocarcinoma. Therefore, it was assumed that among the gene mutation pathways of colon tissue carcinogenesis, APC gene is the mutation that occurs at an earlier period, and that KRAS and TP53 are important gene mutations following APC gene mutations during the progression of colon cancer (4; 10). Compared with normal tissues, adenoma, and adenocarcinoma tissues, only mucinous adenocarcinoma tissues had KRAS and TP53 mutations, and this variation of genetic behavior of mutations may be associated with tumor heterogeneity. Therefore, based on our finding of different genotypic mutation status at two different malignancy locations, we should consider KRAS gene testing for multiple primary malignancies during clinical practice. Previous studies have shown that KRAS has a significant impact on colorectal cancer malignancy. Current NCCN guidelines have indicated that cetuximab is ineffective for colon cancer with KRAS-mutant. Therefore, clarifying the mutant genotype of the primary tumor is critical to improving patient outcomes.

With the current advances in colon cancer research, we have found a gradual increase in the incidence of right-sided colon cancer and a decrease in the incidence of left-sided colon cancer (11; 12). The correlation between tumor location and colon cancer prognosis has been explored for decades based on the differences between the left-sided and right-sided colon cancers. Several studies published in the 1980s showed that the location of the tumor had no effect on the overall survival rate (13; 14). Several studies have proposed that the difference in prognosis between the left-sided and the right-sided colon cancer may be attributable to environmental, genetic, and embryonic factors (15). In the treatment of patients with metastatic colon cancer, different types of targeted agents are used for the different primary tumor sites (16). Therefore, exploring the underlying causes affecting the left-sided and right-sided colon cancer has profound implications for improving the prognosis of patients. Current researches have suggested that patients with left-sided colon cancer have better prognosis than those with right-sided colon cancer (17–19). However, the reason for the difference in survival between patients with left-sided

and right-sided colon cancer is currently unknown. It was hypothesized that differences in the embryonic origin, fecal exposure, and the time of detection of colon cancer at different sites may be the main reasons leading to this outcome (20). The midgut during the embryonic stage develops into the right hemicolon extending from the cecum to nearly two-thirds of the transverse colon, with blood supply from the superior mesenteric artery. In contrast, the hindgut during the embryonic stage develops into the left hemicolon that extends from one third of the distal transverse colon to the upper anal canal, with blood supply from the inferior mesenteric artery. We can therefore hypothesize that the difference in the prognosis between the right-sided and the left-sided colon cancer may be related to genetic and environmental factors. However, no study has revealed the underlying cause of the difference in prognosis between the right-sided and left-sided colon cancer. In the present study, multiple malignant tumors at different states were obtained from the environmental system of the same organism. The number of somatic gene mutations in the right-sided colon cancer mucinous adenocarcinoma was higher than that in the left-sided colon cancer mucinous adenocarcinoma in case 2, but the number of gene mutations and the types of mutated genes were identical when comparing the normal tissues of the two sites. And, the number of somatic mutations in the right-side colonic adenocarcinoma was less than the left-side colonic mucinous adenocarcinoma in case1. Based on these results, we can conclude that the poorer prognosis of the right-sided colon cancer is not significantly related to its underlying genetic variant level. Compared with the right-sided colon cancer, the left-sided colon cancer patients, especially patients with sigmoid colon cancer, are more likely to develop symptoms of incomplete intestinal obstruction at an early stage because of the smaller intestinal lumen and narrower tumor type, making the symptoms of onset easier to detect, which results in relatively small in tumor growth and relatively early in stage at the time of medical examination and diagnosis. With the delay in the time of consultation, the possibility of more somatic gene mutations increases in patients with right-sided colon cancer, and the malignancy of the right-sided colon tumor worsens, thus leading to worse prognosis.

Similarly, this research center performed a large statistical analysis of the prognostic differences between approximately 2400 patients with left-sided and right-sided colon cancer. The results showed (Fig. 4, 5): there were more T3-4 patients in right-sided colon cancer with relatively late stages; and the prognosis of patients with right-sided colon cancer was worse compared to the left-sided colon cancer. This was also consistent with the hypothesis of this study: the longer growth cycle of the right-sided colon cancer led to worse pathological staging, malignancy, and prognosis.

Active signaling via the EGFR pathway is more common in left-sided colon cancer, ostensibly making it more sensitive to the EGRF inhibitor-based therapy (21; 22). Both the NCIC CTG CO.17 retrospective trial report as well as the study by Wang et al. have demonstrated significant ORR, PFS, and OS in patients with left-sided colon cancer upon the addition of cetuximab to first- or second-line chemotherapy in patients with KRAS wild-type chemo-resistant metastatic colorectal cancer, while only limited benefits was observed in patients with right-sided colon cancer (23; 24). In the current study, the comparison of the key genes in colon cancer at two different sites revealed that the number of exon mutations in KRAS and APC genes were higher in the right-sided colon cancer than in the left-sided colon cancer, which may

suggest that patients with left-sided colon cancer have better prognosis than those with right-sided colon cancer.

In the present study, a small number of cases were chosen, which failed to draw consistent conclusions from a large number of patient data. Moreover, this study only explored the relationship between the number of somatic gene mutations and malignant tumor behavior, and did not address protein expression as well as RNA levels. Only sections of tumor specimens were extracted and sent for second-generation gene sequencing. Due to the heterogeneous nature of tumors, the variants detected using small pieces of tissue may not fully reflect the full range of cellular variants at the lesion site. The lack of control sample analysis in this experiment may make it unable to distinguish somatic mutations from germline mutations.

Conclusion

The number of somatic gene mutations showed an increasing trend during the progression from normal tissue to the most malignant mucinous adenocarcinoma. The prognosis of patients with right-sided colon cancer was worse than that of patients with left-sided colon cancer, and there was no difference in the underlying genetic level of the two types of colon cancer. The longer growth cycle of the right-sided colon cancer may be the main reason for the different prognosis of the two.

Abbreviations

IARC: International Agency for Research on Cancer. APC: adenomatous polyposis coli. KRAS: Kirsten-ras. TP53: tumor protein 53. OS: overall survival. PFS: progress free survival. DFS: disease free survival. MPMs: multiple primary malignancies. NCCN: National Comprehensive Cancer Network.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

The article's topic design was contributed by WW and HQ. Data processing and article writing were performed by WW as a major contributor. XM is the corresponding author. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The ethics review committee of Guangxi Medical University Cancer Hospital approved the present study.

Consent for publication

Written informed consent for this research was obtained from the patient prior to surgery. The patient has provided written permission for the publication.

Competing interests

The authors declare that we have no conflict of interest.

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Tables

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Figures

Figure 1

Frequency of genetic mutations of case 1.

Figure 2

Frequency of genetic mutations of case 2.

Figure 3

Key gene mutations.

Figure 4

The tumor stage of 2400 patients.

Figure 5

The survival curve of 2400 patients.